The association between maternal influenza, drug consumption and oral clefts

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Saxén, I. The association between maternal influenza, drug consumption and oral clefts. *Acta Odont. Scand.* 33, 259—267, 1975.

The linkage between potentially teratogenic factors was studied in a material of 599 children with oral clefts and their matched controls. A method based on Yule's Q coefficient describing the degree of association between two dichotomous variables was applied. All factors studied (five groups of drugs taken by the mothers during early pregnancy, maternal influenza and fever) were significantly associated with the birth of children with clefts. The only factor whose association with clefts was explained by linkage to other factors was fever. In addition, the association between clefts and antipyretic analgesics other than salicylates could be partly explained by controlling the intake of salicylates. Although there was a strong association between influenza and consumption of salicylates, the correlation of neither of the two factors with oral clefts could be even partly explained by controlling the other. The method is considered suitable for epidemiological studies of congenital defects.

Key-words: Cleft lip; cleft palate; abnormalities, drug-induced; drugs; influenza; epidemiologic methods; teratology

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When the etiology of congenital defects is studied epidemiologically, the most difficult problem is perhaps the linkage between different potential teratogens. A relationship found between a certain factor and the birth of malformed children may be explained by some other factor, which is associated with the first factor as well as with the defects. Thus, when the possible causality of an association is evaluated, the impact of confounding factors must be taken into account (*Slone et al.*, 1973).

When the etiological role of drugs consumed by pregnant women is studied, obvious confounding is created by the pathological conditions for which the drugs were taken, and also by other drugs which were taken simultaneously. Typical examples of these complex situations are the linkages between hormone intake and maternal bleeding (*Yerushalmy*, 1972), between antiemetics and vomiting (*Richards*, 1972), and between epilepsy and anticonvulsant therapy (*South*, 1972). However, the factor pair that has been by far most frequently discussed in this connection is influenza and the intake of analgesics (see Discussion). Yet, although the problem of intercorrelations between these factors has been discussed in most papers dealing with the possible teratogenicity of drugs or illnesses, the role of confounding has only seldom been examined.

In the present study an attempt was

Received for publication, June 11, 1975.

made to analyse the linkages between influenza and fever, and drugs taken during pregnancy, which were all significantly associated with the birth of children with oral clefts. A survey analysis method presented by *Davis* (1971) was applied; the method is based on the Q coefficient (*Yule*, 1912).

The material consisted of 599 children with oral clefts and their matched controls. The drug consumption of the mothers of these children has been studied earlier, and during the 1st trimester the intake of several drugs was significantly more frequent among mothers of children with clefts than among controls (*Saxén*, 1975).

MATERIAL AND METHODS

Full details of the method used in the present study are presented by *Davis* (1971). In the following the basic principles of the method will be shortly presented, and its application to the present study described.

a) Yule's Q coefficient and survey analysis

Yule's Q coefficient describes the degree of association between two dichotomies, i.e. two two-category variables. Q is calculated from a four-fold table where the two dichotomies, X-Not X and Y-Not Y are cross-tabulated (Table II). Q_{XY} equals . 00 when X and Y are independent, and has a maximum of 1.00 for the strongest (possible) positive and a minimum of —1.00 for the strongest (possible) negative association. Q is insensitive to multiplication of row or column frequencies by a positive constant. Confidence limits can be calculated for Q at different levels of significance. When both limits have the same sign, Q is said to be significant.

A third variable, e.g. a confounding variable (C), can be introduced to test the properties of Q_{XY}. (Additional confounding variables can also be introduced, but the material of the present study was too small to allow an analysis with more than three variables). The data of the analysis using three variables is presented in an eight-fold table composed of two four-fold XY correlation tables, differing in their category of C (Table III). Q_{XY} can be calculated separately for these two conditional tables. The conditional Q's thus describe the degree of association between X and Y when C has a given value.

The partial association coefficient, $Q_{XY:TIED\ C'}$ is obtained by combining the two conditional coefficients. The partial is thus the $Q_{XY'}$ where C is controlled. (The partial is a weighted average of the conditional coefficients).

Correspondingly, a differential association coefficient, $Q_{XY:DIFF\ C'}$ is obtained by combining the Q coefficients recalculated among subgroups of cases that are different in their category of C. The original Q_{XY} coefficient is called the zero-order coefficient, because zero additional variables have been controlled or made to differ. Q_{XY} is a weighted average of the partial and differential Q's.

The effect of a confounding variable on the relationship of two variables is studied by comparing the different Q coefficients with each other. When seeking a confounding variable to explain Q_{XY}, i.e. the relationship between X and Y, the variable should have a zero-order association with both X and Y, and these associations should, in general, be stronger than Q_{XY}.

The indications of certain combinations of different Q values are as follows: If the partial coefficient, $Q_{XY:\ TIED\ C'}$ does not differ from the zero-order coefficient, $Q_{XY'}$ it is concluded that the relationship between X and Y exists, whether C is

controlled, varies, or is ignored. If the partial is negligible, i.e. between —0.10 and +0.10, it can be said that X and Y are not related, when C is controlled, and that C thus explains the relationship between X and Y.

If the partial is less than the zero-order coefficient, but still non-negligible (more than 0.10), it can be said that C partly explains the XY-relationship, since the value of Q_{XY} can be lessened by controlling for C, but C does not, however, account for the XY relationship. If the partial is stronger than the zero-order or has even an opposite sign, C is called a suppressor variable, since the relationship between X and Y is strengthened when C is controlled.

The difference between the partial and differential coefficients can also be examined when the effect of C on the XY relationship is evaluated. If it is less than 0.10 units, the XY relationship remains, whether C is controlled or not. If it is 0.10 units or more, C is either a suppressor variable or C explains (some of) the XY relationship, depending on the values of the coefficients. If the two conditional coefficients, calculated from the two fourfold tables differing in their category of C, differ greatly from each other, in general more than 0.10 units, C is said to cause a specification of the XY relationship, because the level of C determines the degree of association between X and Y (Table III).

b) Material of the study and application of the method

The material of the present study consisted of all oral clefts reported to the Finnish Register of Congenital Malformations during the years 1967—1971, altogether 599 children, and a matched control group of 590 children. The controls were matched for the time of birth and place of residence. Since the control and study mothers also had visited the same Maternity Centers during pregnancy, some matching could be expected to have occurred for the factors studied in the present study. The extent of this matching was checked by comparing the relative risks calculated as in a matched-sample study and as in a random-sample study (Table I). The small differences indicated only suggestive matching, which was considered too slight to have an influence on the results of the present analysis.

Table I. *An analysis of the possible effect of the seasonal and geographical matching on matching the potential teratogens studied. Relative risks calculated as in a matched-pair analysis and as in a random-sample study*

	Relative risk	
	Matched-pair analysis	Random-sample study
Salicylates	3.29	2.96
Other antipyretic analgesics	1.89	1.76
Opiates	3.42	3.40
Penicillins	2.14	1.97
Fever	1.58	1.57
Influenza	2.00	1.96

A detailed description of the organization of the Register has been given elsewhere (*Saxén, Klemetti & Härö*, 1974), and the basis of the present material has also been described earlier (*Saxén & Lahti*, 1974). The frequency of drug consumption among the mothers of these children has been analysed in a recent paper (*Saxén*, 1975), where the percentages of mothers taking those drugs that are analysed in the present paper can be found. The cross-tabulations that were performed for the calculation of the Q coefficients are not presented here

because of their large number. Only the most interesting of them, namely those concerning salicylates and influenza are presented in the following description of the course of the analysis.

Information on »influenza» was mostly based on maternal information, and hence on the mothers' own definitions about an acute infection with fever. Because the prevalence of serologically detected influenza in Finnish pregnant women has been reported to be of the same order as in the present material (*Pyhälä & Aho*, 1975), most of the »influenza» in the present study can be expected to have been caused by influenza virus.

The key variable Y (= the variable to be explained) was here the state of the child, i.e. no cleft vs. cleft (control-study). As predictor variables (X) potential teratogens were selected, which had been previously shown to be associated with oral clefts: influenza, fever and four groups of drugs. In order to get a sufficient number of cases for the cells of the eight-fold tabulations, two additional variables were formed: three groups of analgesic drugs were combined, as well as the groups of influenza and fever. Dichotomies were formed of all variables, the dichotomy being »drug consumption/febrile illness *reported* during the 1st trimester», vs. »drug consumption/febrile illness *not reported* during the 1st trimester». Four-fold tables were run, from which Yule's Q coefficients were calculated for all predictor variables (X) and Y, representing thus the associations between the potential teratogens and oral clefts.

Table II is an example of such a table, giving the number of control and study mothers who reported influenza during the 1st trimester, and the Q_{XY} calculated from this table. Mothers who reported influenza during pregnancy, but not the time of attack, were considered inapplicable, and were excluded from the tabulations. Similar four-fold tables were run also for all pairs of potential teratogens, and the zero-order associations $Q_{X_iX_s}$ were calculated, which thus describe the degree of linkage between different

Table II. *A four-fold table giving the number of control and study mothers (Y) reporting influenza during the 1st trimester (X). Yule's Q coefficient Q_{XY} is calculated from the table*

Y \ X	Influenza Yes	No
Control	34	552
Study	64	527

N = 1177
inapplicable 12
total N = 1189
Q_{XY} = 0.33*

($p < 0.025$, confidence limits at the 95 % level 0.13 and 0.53)

Table III. *An eight-fold table giving the number of control and study mothers (Y) reporting influenza during the 1st trimester (X) grouped according to reporting of salicylate intake during the 1st trimester (C). The partial, $Q_{XY:TIED\ C}$ differential, $Q_{XY:DIFF\ C}$ and the two conditional coefficients calculated from this Table are presented*

C Salicylates	Y \ X	Influenza Yes	No	Conditional Q's
Yes	Control	7	25	$Q_{XY:C}$ = 0.04
	Study	18	69	
No	Control	20	435	$Q_{XY:Not\ C}$ = 0.40
	Study	38	357	

N = 969
inapplicable = 220
total N = 1189
$Q_{XY:TIED\ C}$ = 0.38
$Q_{XY:DIFF\ C}$ = 0.39

Table IV. *A percentual table compiled from the figures of Table III, giving the percentage of mothers reporting influenza in the groups with and without reported intake of salicylates*

Salicylates	Influenza	
	Control %	Study %
Yes	21.8	20.7
No	4.4	9.6
Total	5.6	11.7

potential teratogens. Confidence limits were then calculated for each Q at the 95 % level.

Eight-fold tabulations were then run with selected confounding factors, and the two conditional Q's as well as the partial and differential coefficients were calculated. Table III is an example of an eight-fold table, which gives the number of control and study mothers (Y) reporting influenza (X) grouped according to their reporting of salicylate intake (C). The partial, differential and conditional coefficients calculated from this table are given. From this table, therefore, the effect of salicylate intake on the association between influenza and oral clefts can be analysed. In order to illustrate the effect of specification of the XY relationship by a certain confounding variable, percentual tables were compiled from the eight-fold tables (Table IV).

RESULTS

The degree of association between oral clefts, drug intake and febrile illnesses is illustrated in Table V. It can be seen that all of the factors studied are significantly associated with clefts and that the strongest associations are found between clefts and the intake of opiates (0.54) and salicylates (0.51). As could be expected, there were strong interassociations between the different potential teratogens studied. These associations were also statistically significant, except two of them, namely those between antipyretic analgesics and fever and between antipyretic analgesics and the combined group of fever and influenza (Table V).

Results of the analysis of the three-variable relationships, i.e. analysis of the impact of confounding factors on the relationship between a potential teratogen and clefts, are presented in Table VI and summarized in Table VII. As confounding variables only such factors were selected which had a distinct association both with the potential teratogen under study and with oral clefts. In addition, restriction of the number of confounding variables used was created by lack of sufficient number of cases in the cells of the eight-fold tabulations.

As can be seen in Table VI, controlling for either opiates or influenza did not lessen the degree of association between salicylates and clefts (the partial coefficients were of the same order as the original zero-order relationship between oral clefts and the intake of salicylates). Thus the association between salicylates and clefts could be explained neither by the intake of opiates nor by influenza. But opiates as well as influenza caused a specification of the relationship, so that among those mothers who took opiates or reported influenza, the association between salicylates and clefts was less evident ($Q_{XY:C} = 0.15$) than among those who did not take opiates ($Q_{XY:NOT\ C} = 0.49$) or did not report influenza ($Q_{XY:NOT\ C} = 0.54$).

In contrast, the degree of association between the intake of antipyretic analgesics could be lessened from 0.27 to 0.15 by controlling for salicylates, which therefore

Table V. *Associations between potential teratogens and oral clefts, Q_{XY}, and interassociations between different potential teratogens, $Q_{X_iX_s}$.*

	Q_{XY}	$Q_{X_iX_s}$							
	ORAL CLEFTS	Salicylates	Other antipyretic analgesics	Opiates	All analgesics	Penicillins	Fever	Influenza	Febrile illness
Salicylates	0.51*		0.79*	0.84*		0.34*	0.76*	0.57*	0.67*
Other antipyretic analgesics	0.27*	0.79*		0.92*		0.51*	0.34	·	0.24
Opiates	0.54*	0.84*	0.92*			0.64*	0.62*	0.64*	0.64*
All analgesics	0.49*					0.52*	0.73*	0.62*	0.66*
Penicillins	0.34*	0.34*	0.51*	0.64*	0.52*		0.65*	0.62*	0.67*
Fever	0.23*	0.76*	0.34	0.62*	0.73*	0.65*		0.90*	
Influenza	0.33*	0.57*	·	0.64*	0.62*	0.62*	0.90*		
Febrile illness	0.26*	0.67*	0.24	0.64*	0.66*	0.67*			

* = $p < 0.025$ (same sign of both confidence limits at 95 per cent level)
= smallest cell expectation in the four-fold table less than 5, Q not calculated.

Table VI. *Three variable results on the impact of different confounding variables (C) on the association between a potential teratogen (X) and oral clefts (Y)*

Potential teratogen X	Q_{XY}	Confounding factor C	Q_{CX}	Q_{CY}	Partial $Q_{XY:TIED\ C}$	Differential $Q_{XY:DIFF\ C}$
Salicylates	0.51	Opiates	0.84	0.54	0.48	0.59
		Influenza	0.57	0.33	0.54	0.46
Other antipyretic analgesics	0.27	Salicylates	0.79	0.51	0.15	0.38
		Opiates	0.92	0.54	0.28	0.28
Opiates	0.54	Salicylates	0.84	0.51	0.51	0.60
		Antipyretics	0.92	0.27	0.73	0.31
		Febrile illness	0.26	0.64	0.65	0.40
Penicillins	0.34	Salicylates	0.34	0.51	0.31	0.46
		Analgesics	0.52	0.49	0.33	0.53
		Influenza	0.62	0.33	0.42	0.09
		Fever	0.65	0.23	0.34	0.36
Influenza	0.33	Salicylates	0.57	0.51	0.38	0.39
		Analgesics	0.62	0.49	0.37	0.39
		Penicillin	0.62	0.34	0.40	0.08
Fever	0.23	Salicylates	0.76	0.51	0.04	0.40
		Analgesics	0.73	0.49	—0.02	0.36
		Penicillin	0.65	0.34	0.20	0.31
		Influenza	0.90	0.33	0.06	0.38

Table VII. *Summary of the analysis of associations between potential teratogens and oral clefts*

Potential teratogen (X)	Degree of association between X and oral clefts (Q_{XY})	Effect of confounding factor (C) on Q_{XY} (Partial association coefficient, $Q_{XY:TIED\ C'}$ in parentheses)		
		explains	explains partly	causes specification or has no effect
Salicylates	0.51			opiates (0.48) influenza (0.54)
Other antipyretic analgesics	0.27		salicylates (0.15)	opiates (0.28)
Opiates	0.54			salicylates (0.51) antipyretics (0.73) febrile illness (0.65)
Penicillins	0.34			salicylates (0.31) analgesics (0.33) influenza (0.42) fever (0.34)
Influenza	0.33			salicylates (0.38) analgesics (0.37) penicillins (0.40)
Fever	0.23	salicylates (0.04) analgesics (—0.02) influenza (0.06)		penicillins (0.20)

explain partly the relationship. Opiates did not affect this association.

The strong association between clefts and the intake of opiates was not lessened when the other factors were controlled. Salicylates had no effect on the relationship, but when the other antipyretic analgesics or influenza were controlled, the association between opiates and clefts increased.

The relationship between penicillin consumption and clefts could not be explained by other factors either. Salicylates, the combined group of analgesics and fever had no effect, whereas influenza caused a clear specification of the relationship. Among mothers who reported influenza there was namely a negative association (—0.32) between penicillins and clefts, whereas among those with no influenza the association was positive (0.44).

Similarly, penicillins specified the association between influenza and clefts, but did not explain it. A specification was also caused by salicylates (Table IV) and the combined group of analgesics, so that among mothers who reported intake of analgesics, there was no association between influenza and malformations. However, the intake of salicylates or analgesics in general did not explain the association between influenza and clefts.

In contrast, the association between fever and clefts became negligible, when salicylates, all analgesics or influenza were controlled. Thus, these factors could explain the association. Penicillins caused

a specification but did not otherwise affect the relationship between fever and clefts.

DISCUSSION

After the influenza epidemic in 1957 an increase in the rate of malformations, particularly the central nervous system defects, was reported in several papers (*Coffey & Jessop*, 1959, 1963; *Saxén et al.*, 1960; *Hardy et al.*, 1961; *Leck*, 1963; *de Groot*, 1967). *Leck* (1963, 1971) observed an increase in particularly cleft lip without cleft palate after the 1957 epidemic as well as after subsequent outbreaks of influenza. However, several authors have not noted alterations in the malformation incidence after influenza epidemics (*Doll, Hill & Sakula*, 1960; *Walker & McKee*, 1959; *Hewitt*, 1962; *Widelock, Csizmas & Klein*, 1963; *Czeizel et al.*, 1967; *Wilson & Stein*, 1969). In these reports, as in some others as well, it has often been suggested that influenza might not be teratogenic, but that some other factors, particularly some drug(s), could be the causative agent, and would thus explain the associations found between influenza and malformations.

That drugs, rather than influenza, were the teratogenic agents, was also suggested by *Leck* (1964) to explain his observations that the incidence of malformations, including cleft lip, was highest after the smallest outbreak of influenza and lowest after the most extensive epidemic. An association between maternal influenza and drug consumption has been demonstrated in several studies on congenital defects (*Richards*, 1972; *Hakosalo & Saxén*, 1971; *Karkinen-Jääskeläinen & Saxén*, 1974) and also in these reports drugs, especially the salicylates, have been suspected of being the teratogenic factor.

The present study showed once again that there are marked interassociations between the drugs taken by the mothers and febrile illnesses, and that confounding is thus an important phenomenon in epidemiological studies. However, here the associations between oral clefts and most of the factors studied could not be explained by confounding created by any of the factors studied. Only the association of fever with clefts disappeared when the intake of drugs was controlled. Also the relationship between clefts and antipyretic analgesics, other than salicylates, was partly explained by the intake of salicylates. The degree of the associations between oral clefts and the other drugs studied, namely salicylates, opiates and penicillins, was, however, not lessened when other factors were controlled. Neither could the relationship between influenza and clefts be even partly explained by the intake of salicylates or other drugs. Thus, the possibility that influenza itself might be teratogenic could not be eliminated in the present study.

The etiological role of the various drugs was discussed earlier and the limitations of the partly retrospective study were also considered (*Saxén*, 1975). When the results of this special analysis are evaluated, some obvious shortcomings should be borne in mind. Only a few selected confounding factors were tested, although several other factors might have affected the relationships and could have been studied. In addition, there are evidently factors presently unknown, which might have an impact on some of the associations. Secondly, in spite of the relatively large material (altogether 1189 mothers), the expected cell frequencies in the eight-fold tabulations were often small, because only a low proportion of mothers reported drug intake or febrile

illnesses during the 1st trimester. Thus conclusions concerning the impact of a confounding factor on the association between a potential teratogen and clefts should be drawn cautiously. The present study, however, showed that the survey analysis method applied here can be used for the analysis of linkages between different possible etiological factors in an epidemiological study of congenital malformations.

Acknowledgements. This work was supported by the National Research Council for Medical Sciences, Finland, and by the Emil Aaltonen Foundation.

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